Date of Deposit: February 20, 2007

Attorney Docket No. 21465-508 UTIL

**AMENDMENT** 

Please amend the claims as follows, without prejudice:

In the Claims:

1. (Currently Amended) A method for amplifying one or more nucleic acids onto a bead

comprising the steps of:

(a) forming a water-in-oil emulsion to create a plurality of aqueous microreactors wherein at

least one of the microreactors comprises a single nucleic acid template, a single bead with a

plurality of molecules of a first primer species disposed thereon, wherein the first primer species

is capable of binding to the nucleic acid template, and amplification reaction solution containing

comprising a plurality of molecules of the first primer species and a second primer species and

reagents necessary to perform nucleic acid amplification, wherein a concentration of the second

primer species is substantially greater than that of the first primer species in the reaction solution;

(b) amplifying the nucleic acids template in the microreactors using the first and second

primer species to form amplified copies of said a complementary nucleic acids, wherein

substantially all of the molecules of the first primer species in the reaction solution are depleted;

and

(c) binding the amplified capturing said copies to on the bead beads in the microreactor

microreactors using the first primer species.

2. (Original) The method of claim 1, wherein a majority of the microreactors include a single

nucleic acid.

3. (Currently Amended) The method of claim 1, wherein said amplification reaction solution is

a polymerase chain reaction solution further comprising nucleotide triphosphates, a

thermostable polymerase, and nucleic acid primers suspended in a buffer compatible with

polymerase chain reaction conditions.

4. (Canceled).

5. (Canceled).

6. (Original) The method of claim 1, wherein said emulsion additionally contains emulsion

stabilizers.

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7. (Original) The method of claim 6, wherein said emulsion stabilizers are selected from the group consisting of Atlox 4912, Span 80, and combinations and mixtures thereof.

- 8. (Original) The method of claim 1 wherein said emulsion is heat stable.
- 9. (Original) The method of claim 8 wherein said emulsion is heat stable to 95°C.
- 10. (Original) The method of claim 1, wherein amplification is carried out by a method selected from the group consisting of transcription-based amplification, rapid amplification of cDNA ends, continuous flow amplification, and rolling circle amplification.
- 11. (Original) The method of claim 1, wherein the emulsion is formed by the dropwise addition of the nucleic acid templates, beads, and amplification reaction solution into an oil.
- 12. (Original) The method of claim 1, performed with at least 10,000 nucleic acids.
- 13. (Original) The method of claim 1, performed with at least 50,000 nucleic acids.
- 14. (Original) The method of claim 1, wherein the microreactors have an average size of 50 to 250 μm in diameter.
- 15. (Original) The method of claim 1, wherein after step (c) each bead captures more than 10,000 amplification copies of a nucleic acid template.
- 16. (Withdrawn) A library comprising a plurality of nucleic acid molecules, wherein each nucleic acid molecule is separately immobilized to a different bead, and wherein each bead comprises over 1,000,000 clonal amplification copies of each nucleic acid molecule, wherein the library is contained in a single vessel.
- 17. (Withdrawn) The library of claim 16, wherein the nucleic acid molecules are selected from the group consisting of genomic DNA, cDNA, episomal DNA, BAC DNA, and YAC DNA.
- 18. (Withdrawn) The library of claim 16, wherein the genomic DNA is selected from the group consisting of animal, plant, viral, bacterial, and fungal genomic DNA.
- 19. (Withdrawn) The library of claim 18, wherein the genomic DNA is human genomic DNA or human cDNA.
- 20. (Withdrawn) The library of claim 16, wherein the bead has a diameter of 2 microns to 100 microns.

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21. (Withdrawn) The library of claim 16, wherein the bead is a sepharose bead.

22. (Currently Amended) A method for amplifying a nucleic acid onto a bead comprising the

steps of:

(a) providing a nucleic acid template to be amplified;

(b) providing a solid support material comprising a generally spherical bead having a

diameter about 10 to about 80 µm, wherein the bead comprises a plurality of molecules of a first

primer species disposed thereon is capable of binding to the nucleic acid template;

(c) mixing the nucleic acid template and the bead in an amplification reaction solution

containing comprising a plurality of molecules of the first primer species, a second primer

species and reagents necessary to perform a nucleic acid amplification reaction in a water-in-oil

emulsion, wherein a concentration of the second primer species is substantially greater than that

of the first primer species in the reaction solution;

(d) amplifying the nucleic acid template using the first and second primer species to form

amplified copies of a complementary said nucleic acid template, wherein substantially all of the

molecules of the first primer species in the reaction solution are depleted; and

(e) binding the amplified capturing said copies to on the bead using the first primer species.

23. (Withdrawn) A kit for conducting nucleic acid amplification of a nucleic acid template

comprising:

(a) a nucleic acid capture bead;

(b) an emulsion oil;

(c) one or more emulsion stabilizers;

(d) instructions for performing the method of claim 1 or claim 22.

24. (Original) The method of claim 1 or claim 22 further comprising the step of enriching for

beads which bind amplified copies of the nucleic acid away from beads to which no nucleic

acid is bound, the enrichment step selected from the group consisting of affinity purification,

electrophoresis and cell sorting.

25. (Original) The method of claim 24 wherein the enrichment step is performed by affinity

purification with magnetic beads that bind nucleic acid.

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26. (Original) The method of claim 1 or 22, wherein at least 100,000 copies of each target nucleic acid molecule are bound to each bead.

- 27. (Original) The method of claim 1 or 22, wherein at least 1,000,000 copies of each target nucleic acid molecule are bound to each bead.
- 28. (Original) The method of claim 1 or 22, wherein between at least 1 to 20,000,000 copies of each target nucleic acid molecule are bound to each bead.
- 29. (Original) The method of claim 1 or 22, wherein the beads are sepharose beads.
- 30. (Original) The method of claim 1 or 22, wherein amplified copies are bound to the beads by a binding pair selected from the group consisting of antigen/antibody, ligand/receptor and polyhistidine/nickel.
- 31. (Original) The method of claim 30, wherein the binding pair is avidin/biotin.
- 32. (Original) The method of claim 25, further comprising the steps of: separating the template carrying beads and magnetic bead; and removing the magnetic beads with a magnetic field.
- 33. (Original) The method of claim 32, wherein the separating is achieved by incubation at a temperature greater than 45°C or by incubating the template carrying beads and the magnetic beads in a solution with a basic pH.
- 34. (Currently Amended) A method for producing a clonal population of nucleic acids, comprising:
- (a) providing a plurality of nucleic acid templates from 50-800 bp in length and beads <u>each</u> <u>comprising a plurality of molecules of a first primer species disposed thereon</u> capable of binding to the nucleic acid templates;
- (b) mixing the nucleic acid templates and the beads in a biological reaction solution e<del>ontaining</del> that comprises a plurality of molecules of the first primer species, a plurality of molecules of a second primer species and reagents necessary to amplify the nucleic acid templates, wherein a concentration of the second primer species is substantially greater than that of the first primer species in the reaction solution;

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(c) forming an emulsion to create a plurality of microreactors comprising the nucleic acid templates, beads, and <u>the</u> biological reaction solution, wherein at least one of the microreactors comprises a single nucleic acid template and a single bead encapsulated in the biological reaction solution, wherein the microreactors are contained in the same vessel.

- 35. (Withdrawn) The method of claim 34 further comprising transcribing and translated the nucleic acids to generate at least 10,000 copies of an expression product.
- 36. (Withdrawn) The method of claim 35, wherein said expression product is bound to said beads by a binding pair selected from the group consisting of antigen/antibody, ligand/receptor, 6Xhis/nickel-nitrilotriacetic acid, and FLAG tag/FLAG antibody binding pairs.
- 37. (Withdrawn) The method of claim 35, wherein the method produces a clonal population of proteins.
- 38. (Withdrawn) The method of claim 37, wherein the proteins are selected from the group consisting of antibodies, antibodies fragments, and engineered antibodies.
- 39. (Withdrawn) An emulsion comprising a plurality of thermostable microreactors, wherein the microreactors are 50 to 200 μm in diameter and comprise a biological reaction solution..
- 40. (Withdrawn) The emulsion of claim 39, wherein the biological reaction solution comprises reagents for performing polymerase chain reaction amplification.
- 41. (Withdrawn) The emulsion of claim 39, wherein the biological reaction solution comprises reagents for performing coupled transcription and translation reactions.
- 42. (Withdrawn) The emulsion of claim 40 or claim 41, wherein the plurality of microreactors comprise a nucleic acid template.
- 43. (Withdrawn) The emulsion of claim 42, wherein the plurality of microreactors comprise one or fewer nucleic acid templates.
- 44. (Withdrawn) The emulsion of claim 43, wherein the plurality of microreactors comprise one or fewer beads that bind to the nucleic acid templates.